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STIMULATION BY ADRENALIN OF THE LUMINESCENCE OF DEEP-SEA FISH AND THE CHEMICAL ASPECTS OF THE LUMINESCENCE OF DEEP-SEA SHRIMP

By E. Newton Harvey
Princeton University

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STIMULATION BY ADRENALIN OF THE LUMINESCENCE OF DEEP-SEA FISH*

By E. Newton Harvey
Princeton University

Collecting deep sea animals in good living condition is very difficult. Whether affected by change in pressure, or temperature, or asphyxiation in the bottles at the end of the nets, it is unfortunately true that most deep sea forms come up quite dead and motionless. Occasionally they are living.

Through the great kindness of Dr. William Beebe, Director of the Bermuda Oceanographic Expedition of the New York Zoological Society, I have recently had an opportunity of studying the luminescence of a large deep sea fish, *Echiostoma ctenobarba*, two specimens of which, about one foot long, were brought into the laboratory in iced sea water in the living condition. They were caught at eight hundred fathoms.

In *Echiostoma* there is a prominent cheek organ and two rows of large photophores along the ventral and lateral walls besides numerous minute photophores scattered over practically the whole body including the dorsal surface. The cheek organ is partially pink in life and was observed to flash with a decidedly bluish luminescence when the fish was handled, especially when lifted out of the sea water. No other luminescence of any kind could be noted, however, despite the fact that the fish was squeezed and twisted to stimulate it strongly. A hypodermic needle was then inserted but no luminescence additional to that of the cheek organ appeared. However, when a little adrenalin (1 :1000 in physiological salt) was injected with the hypodermic into the side about one-third toward the tail end, there immediately appeared a yellowish luminescence of photophores locally, near the point of injection and soon practically all of the photophores of the fish were luminescing with a yellowish moderately intense continuous glow. This lasted a few minutes and then went out and could not be excited again by rubbing or handling but appeared as before on a second, third and fourth injection of adrenalin. The last injection was of ten minims and excited all or-

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gans and also the pectoral and ventral fins. There is no doubt of the luminescence of these fins despite the fact that they do not possess any marked organs. No luminescence was observed in the tail, anal fins, long pectoral rays, or barbel on lower jaw. The cheek organ flashed at intervals after adrenalin injection but did not change in rhythm or in any noticeable way. The flashing of this organ is not due to unscreening of a continuously luminous surface. The light appears and disappears on the organ itself and for this reason we may presume that *Echiostoma* is self luminous and does not harbor luminous bacteria as is the case in the Dutch East Indian fish, *Photoblepharon* and *Anomalops,* which also possess cheek organs.[1]

There is no doubt of the stimulating action of adrenalin on these photophores. The observations add a second example of luminous fishes known to be excited to luminescence by adrenalin. The first was the California toad-fish, *Porichthys,* described by Greene and Greene.[2] It is a surface form, difficult to stimulate in other ways but which gives a brilliant long lasting glow of its eight hundred odd photophores after injection of adrenalin. The fire-fly also glows continuously and brightly after adrenalin injection.[3]

As the photophores of *Porichthys* receive a very sparse nerve supply, Greene believes that adrenalin acts directly on the photogenic cells. I can state, however, that it does not cause luminescence of the worm, *Chaetopterus*, or of hydroids. In the fire-fly there is considerable evidence of a nerve-muscle mechanism controlling the flash.[4] Studies of the photophore nerve supply of deep sea fish would be very valuable and are much needed.

It should be mentioned that adrenalin is not a stimulant for light production after a fish has been dead some time. Other dead deep sea fish, and even a feebly moving *Linophryne arborifera* could not be made to light by injecting adrenalin. For the physiologist, the great problem is to get the material in good living condition. It is my belief that increased temperature is the chief lethal factor. When we remember that temperatures a mile deep are four to five degrees Centigrade while the

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[1]*Harvey, E. N.* The Production of Light by the Fishes. Photoblepharon and Anomalops. Pub. No. 312 Carnegie Institution Washington, p. 43, 1922. Natural History *25,* 353, 1925.

[2]*Greene, C. W.* and *Greene, H. H.* Phosphorescence of Porichthys notatus, the California Singing Fish. Amer. Jour. Physiol., *70,* 500, 1924.

[3]*Creighton, W. S.* The Effect of Adrenalin on the Luminescence of Fire-flies. Science, *63,* 600, 1926.

[4]*Dahlgren, U.* The Production of Light by Animals. Jour. Franklin Inst. March and May, 1917.

surface water is twenty-five degrees Centigrade, and also that it takes over an hour to haul in the nets, we realize the unfavorable conditions to which these deep sea forms are subject. Perhaps we are lucky to observe luminescence under any circumstances.

CHEMICAL ASPECTS OF THE LUMINESCENCE OF DEEP-SEA SHRIMP*

By E. Newton Harvey
Princeton University

It is convenient to group all luminous organisms into two great classes, those which produce a steady continuous light quite independent of stimulation, the luminous bacteria and the fungi, and those whose luminescence appears only on agitation or stimulation of some kind, including all the others. These may again be divided into forms whose light is intracellular like *Noctiluca* and the fire-fly, and those with extracellular luminescence, forms which secrete a luminous slime or which throw a fluid from glands into the sea water in which they live. Many medusae and the ostracod crustacean, *Cypridina,* belong in the latter group.

Such forms often store up a very large amount of luminous material which they pour out, surrounding themselves with a barrage of fire behind which we may suppose they make their escape from the jaws of some predacious enemy. The most notable and spectacular animal of this class is a small squid, *Heteroteuthis dispar,* found in the Mediterranean and especially at Messina, where I have had the opportunity of studying them. Most of the ink sac has become transformed into a luminous gland. When disturbed, the glowing secretion is shot out thru the siphon as a cloud of luminescence that surrounds the animal. Attacking fish would be subjected to a veritable bombardment of liquid fire quite as startling if not as dangerous as any developed during the war. It is almost paradoxical to find an organ developed for producing the very blackest material, suddenly transformed into one manufacturing not only a clear fluid but a fluid actually shining with its own light.

Such a mechanism of defence must be quite effective, for several other creatures have appropriated the idea. One of these is the deep sea shrimp or prawn, *Systellaspis.* Such forms were first described by Alcock[1] and observed by Beebe[2] during the "Arcturus Ad-

*Contribution, New York Zoological Society Department of Tropical Research, No. 362.
[1]*Alcock, A.* A Naturalist in Indian Seas, p. 134 1902.
[2]*Beebe, W.* The Arcturus Adventure 1927.

venture". Through the kindness of Dr. Beebe, I have recently had an opportunity of making some observations on the chemistry of luminescence in these forms, which have been obtained quite regularly in the tow nets from 600-800 fathoms, about 10 miles south of Nonesuch Island, Bermuda. The shrimp is about 1½ in. long, bright red in color, with a well spiked rostrum, very long antennae and a row of black dots along the sides. These dots are luminous organs although I have never seen light coming from them.

When brought to the surface and placed in iced sea water, since they come from depths where the temperature is about 5 C., they live for several hours, and with well dark adapted eyes one can see that this sea water is aglow with their luminescent secretion, the light lasting for some time. Touch the shrimp with a rod and immediately a cloud of bluish luminescent secretion is shot out from glands near the mouth, and is carried by convection currents thru the sea water.[3]

The luminescence of all organisms is the result of a slow burning or oxidation of a definite compound luciferin, in the presence of an enzyme luciferase. This was first proven to be the case by Dubois in 1886 in the large West Indian elaterid beetle, *Pyrophorus,* later in the mollusc, *Phelus dactylus,* and since then I have found these bodies in lampyrid fireflies, the ostracod crustacean, *Cypridina,* the worm, *Odontosyllis,* and Hickling has described them in the fish, *Malacocephalus laevis.*[4] Curiously enough it is not possible to demonstrate luciferin and luciferase in many of the 40 odd orders of animals containing luminous forms. As the opportunity has appeared I have been studying this point over a period of fifteen years, and table 1 shows the organisms tested and the group to which they belong.[5] Of special interest is the question as to whether the luciferin of one species will react with the luciferase of another. It is not possible to obtain simultaneously all the luminous animals that one would like to test but the ostracod, *Cypridina,* can be dried and its power of luminescence retained indefinitely (at least over a period of 12 years). light appearing whenever the dried animals are moistened. Table 1 shows also how other organisms react with *Cypridina* luciferin and luciferase.

[3]For histology of the organs see Dahlgren, U. The Production of Light by Animals. Journ. Franklin Inst. June 1917.

[4]For Chemistry of luminescence see Harvey, E. N., The Nature of Animal Light 1920; Recent Advances in Bioluminescence. Physiological Reviews *4,* 639, 1924. Bioluminescence. Bull. Nat. Research Council. No. 59, p50, 1927.

[5]*Harvey, E. N.* Additional Data on the Specificity of Luciferin and Luciferase, together with a general survey of this reaction. Am. J. Physiol. *77,* 548, 1926.

The preparation of luciferase and luciferin solutions is very simple. The former is obtained by merely making a cold water extract of the luminous organ, when both luciferin and luciferase dissolve and the luciferin oxidized with luminescence in a short time, leaving the luciferase (an enzyme) behind. Like all enzymes, luciferase is destroyed on boiling, whereas luciferin is not. Consequently luciferin is prepared by making a hot water extract of the luminous organ and cooling. This luciferin solution is quite dark but when mixed with luciferase, also dark, will again produce light.

It was found that *Systellaspis* luciferin mixed with *Systellaspis* luciferase would give a good luminescence, whereas *Systellaspis* luciferin mixed with *Cypridina* luciferase produced no light, nor would *Systellaspis* luciferase give light with *Cypridina* luciferin. This is quite in line with all the previous evidence I have been collecting[5], namely, that the luciferin-luciferase reaction is specific, that luciferin will not react with luciferase of other species belonging to a different group. However, the case of *Systellaspis* is of especial interest, sinse its luminescence is bluish and looks exactly like that of *Cypridina*, and the two forms are Crustacea, fairly closely related. It is the first time I have had the opportunity of testing two orders within the same class.

Only if luminous animals are very closely related, will the luciferin of one species react with the luciferase of another, as two genera of fireflies or two genera of ostracods. In this case an interesting experiment can be carried out where the luminescence of the two species differs in color, as in fire-flies of the genera *Photinus* (reddish luminescence) and *Photuris* (yellowish luminescence). Intermixing luciferin and luciferase of these genera shows that the color of the resulting luminescence is not intermediate but is that of the fire-fly supplying the *luciferase*. This must mean that luciferase is the source of the light. From this and other evidence I have come to the conclusion that the energy for luminescence comes from the oxidation of luciferin.[4] The luciferase plays two roles:—(1) that of an enzyme, accelerating the oxidation of luciferin. (2) to supply molecules which can easily pick up the energy set free in oxidation. Such molecules the chemist call "excited molecules" and their excess energy can be liberated as radiation which we see as the luminescence of the animal. The color (wave-length) of the radiation will depend on the specific chemical configuration of the luciferase mole-

cules, which differ in different species and is so different in different groups that excitation cannot occur at all.

Thus, *Systellaspis* has supplied a very necessary link in our chain of evidence concerning the luciferin-luciferase reaction and I express my sincere thanks to Dr. Beebe, Director of the Bermuda Oceanographic Expedition of the New York Zoological Society for making it possible to obtain these unusual forms.

TABLE I

Group	*Species*	*Place*	*Luciferin-Luciferase reaction*	*Reaction with Cypridina luciferin and luciferase*	*Reported by*
BACTERIA	*Bacillus fisheri*	Princeton	—	—	Harvey
	Photobacterium phosphorescens	Woods Hole	—	—	Harvey
	Photobacterium javanese	Java	+	not tried	Gerretsen
FUNGI	*Panus stipticus*	Woods Hole	—	—	Harvey
SPONGES	*Grantia*	Friday Harbor	—	not tried	Harvey
RADIOLARIA	*Collozoum inerme*	Naples	—	—	Harvey
	Thalassicola nucleata	Naples	—	—	Harvey
CYSTOFLAGELLATES	*Noctiluca miliaris*	Japan	—	—	Harvey
MEDUSAE	*Aequorea forskala*	Friday Harbor	—	—	Harvey
	Mitrocoma cellularia	Friday Harbor	—	—	Harvey
	Pelagia noctiluca	Naples	—	—	Harvey
PENNATULIDS	*Pennatula phosphorea*	Naples	—	—	Harvey
	Cavernularia haberi	Japan	—	—	Harvey
	Ptylosarcus sp. ?	Friday Harbor	—	—	Harvey
CTENOPHORES	*Bolina sp. ?*	Friday Harbor	—	—	Harvey
	Mnemiopsis Leidyi	Woods Hole	—	—	Harvey
	Beroe ovata	Naples	—	—	Harvey
	Eucharis multicornis	Naples	—	—	Harvey
OPHIURIANS	*Amphiura squamata*	Naples	—	—	Harvey
ANNELIDS	*Odontosyllis phosphorea*	Bermuda	+	—	Harvey
	Tomopteris helgolandica	Plymouth	—	—	Harvey
	Polycirrus caliendrium	Plymouth	—	—	Harvey
	Chaetopterus variopedatus	Woods Hole	—	—	Harvey
	Harmithoe imbricata	St. Andrews, N.B.	—	—	Harvey
	Acholoe astericola	Naples	—	—	Harvey
	Misroscolex phosphorea	Naples	—	—	Harvey
OSTRACODS	*Cypridina hilgendorfii*	Japan	+		Harvey Kanda
	Pyrocypris sp. ?	Java	+	+	Harvey
	Cypripina sp. ?	Jamaica, B. W. I.	+	+	Harvey
COPEPODS	*Metridium sp. ?*	Naples	—	—	Harvey
SCHIZOPODS	*Meganyctiphanes norvegica*	St. Andrews, N.B.	—	—	Harvey
DECAPODS	*Acanthephyra sp. ?*	Bermuda	+	—	Harvey
MYRIAPODS	*Geophilus sp. ?*	Java	—*	not tried	Harvey
INSECTS	*Pyrophorus noctiluca*	Cuba	+	not tried	Dubois Harvey
	Luciola viticollis	Japan	+	—	Harvey
	Photinus pyralis	Princeton	+	not tried	Harvey
	Photuris pennsylvanica	Princeton	+	not tried	Harvey
LAMELLIBRANCHS	*Pholas dactylus*	Mediterranean & Plymouth	+	—	Dubois Harvey
CEPHALOPODS	*Watasenia scintillans*	Japan	—	not tried	Harvey
	Heteroteuthis dispar	Messina	—	—	Harvey
ASCIDIANS	*Pyrosoma sp. ?*	Monaco	—	—	Harvey
BALANOGLOSSIDS	*Ptychodera sp. ?*	Bermuda	—	—	Harvey
	Balanoglossus minutus	Naples	—	—	Harvey
FISH	*Photoblepharon palpebratus***	Banda Island	—	—	Harvey
	*Anamalops katoptron***	Banda Island	—	—	Harvey
	*Monacentris japonica***	Japan	—	not tried	Harvey
	Malacocephalus laevis	England	+	not tried	Hickling

*Dilute solutions.
**Contain luminous bacteria.